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One-Pot Four-Enzyme Synthesis of Ketoses with Fructose 1,6-Bisphosphate Aldolases from *Staphylococcus carnosus* and Rabbit Muscle

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Abstract

By the action of D-fructose 1,6-bisphosphate aldolases (FruA) from rabbit muscle and *Staphylococcus carnosus*, various ketoses were synthesized from glyceraldehydes or other aliphatic aldehydes as acceptors in a one-pot four-enzyme system.

Keywords

Enzymatic synthesis; aldolase; ketose; FruA; *Staphylococcus carnosus*

D-Fructose 1,6-bisphosphate aldolase, FruA, catalyzes the pivotal step of glycolysis in vivo, which is the reversible degradation of D-fructose 1,6-bisphosphate to D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP). However, it is worth noting that the equilibrium constant for this reaction strongly favors the synthesis direction, which makes FruA synthetically useful as a biocatalyst.¹ The FruA isolated from rabbit muscle (RAMA) is the most widely investigated DHAP-dependent aldolase for preparative synthesis, due to its commercial availability, high activity, and relaxed aldehyde substrate specificity.^{2–4} Unfortunately, it was reported that RAMA suffered from low operational stability and loss of activity during synthesis.⁵ In contrast, FruA from bacterial sources, such as FruA from *Staphylococcus carnosus* (FruA_{S.car}), is exceptionally temperature and pH stable.^{5–6} In Sheldon's work, the activities and stabilities of two bacterial FruA from *S. carnosus* and *S. aureus* were compared with RAMA to investigate their scope of synthetic application.⁷

While FruA aldolases can tolerate different aldehydes as acceptors, they, like other DHAP-dependent aldolases, are very specific for the donor substrate DHAP. Unfortunately, DHAP

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Supplementary data: Supplementary data (Cloning, expression and purification of FruA_{S.car} and NMR spectra of ketoses) associated with this article can be found, in the online version, at doi:

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is rather expensive and unstable, which greatly hampers their application in large-scale synthesis.^{8–10} A number of chemical and enzymatic approaches have been developed for the preparation of DHAP, however, most of the approaches do not serve as feasible routes to DHAP on a large scale.¹¹ Among the existing routes toward DHAP, the most elegant one is to combine the DHAP synthesis steps with the aldol reaction catalyzed by aldolase. Fessner and co-workers first developed a one-pot system in which DHAP was generated from the oxidation of relatively cheap L-glycerol 3-phosphate (L-GP) by glycerol phosphate oxidase (GPO).¹² In our previous work, we utilized this strategy and developed a one-pot four-enzyme system which can produce sugar molecules directly.^{13–14} Employing this system and through the action of L-rhamnulose 1-phosphate aldolase (RhaD) and L-fuculose 1-phosphate aldolase (FucA), we successfully synthesized four important rare sugars from a much cheaper starting material, racemic DL-glycerol 3-phosphate (DL-GP).^{13–14} In the present study, both RAMA and FruA_{S.car} were studied in the preparative synthesis of ketoses using the one-pot four-enzyme system (Scheme 1).

To obtain the enzyme FruA_{S.car}, the gene *fda* encoding FruA from *S. carnosus* was amplified by PCR using plasmid pKKfda as the template. The *fda* gene amplified was digested with BamHI and XhoI then ligated into the pET-28a plasmid with the same enzymes digested. The recombinant plasmid pET28a-fda was then transformed into *Escherichia coli* BL21 (DE3) to express FruA_{S.car} and the target protein was purified by Ni²⁺-NTA column (Figure 1) (see Supplementary Information for detailed procedures of cloning and purification of FruA_{S.car}).

To investigate the stereoselectivity of FruA_{S.car} toward D- and L-glyceraldehydes, D-fructose and L-sorbose were synthesized accordingly through the one-pot system. Unlike RhaD and FucA, which generate a mixture of diastereomers in our previous study and the stereoselectivities of which were influenced by the configuration of acceptors,^{13–14} FruA_{S.car} demonstrated excellent stereoselectivity toward D- and L-glyceraldehyde. Only a single product was isolated for each reaction (D-fructose and L-sorbose respectively). Notably, FruA_{S.car} seems to display a discrimination towards D- and L-glyceraldehydes since a much higher yield was obtained for D-glyceraldehyde reaction (60%) compared with the L-isomer (28%) (Table 1). It is highly possible that the 2(R)-hydroxy of the aldehyde acceptor is favorable for enzyme recognition and thus lower yield was observed for L-glyceraldehyde.¹⁵ This hypothesis could be further evidenced by the low yields obtained when aliphatic aldehydes (absence of 2-hydroxy) were used as acceptors (Table 1).

As previously reported, FruA has a flexible substrate specificity and accepts various aldehydes. We thus proceeded to utilize this property and successfully synthesized a number of ketoses using simple aliphatic aldehydes as acceptors in the one-pot reaction system (Table 1). The results clearly indicate that the yields are quite similar for reactions of which acetaldehyde, propionaldehyde, and butyraldehyde are acceptors. Meanwhile, it demonstrates that FruA_{S.car} and RAMA have very similar catalytic activities. A possible explanation could be that RAMA displays comparable pH and temperature stability as FruA_{S.car} under the reaction conditions. However, when pentanaldehyde was used as the acceptor, a decreased yield was observed because of its poor water solubility. Thus a cosolvent DMSO at 10% (v/v) concentration was added into the reaction mixture to enhance its solubility. It is worth noting that the generally low yields for all aliphatic aldehydes are probably owing to the absence of hydroxyl groups compared with glyceraldehydes. Less favorable interactions resulted in lower yields because it was reported earlier that aldehydes which carry a hydroxyl function had higher affinity to and correct recognition by the biocatalyst, and formed thermodynamically stable cyclic products.¹⁵

Furthermore, the stereoselectivities of FruA_{S.car} and RAMA are both very high, as the configuration of vicinal diols produced from the aldol addition with aliphatic aldehydes follows that of the glyceraldehydes: only one diastereomer was isolated for each reaction and the products were verified by NMR (see Supplementary Information).^{7,16–18} These results were consistent with Sheldon's work, although they directly used DHAP as the donor and preparative scale synthesis was only achieved with butanal.⁷

In summary, we proceeded with the one-pot four-enzyme system and synthesized different ketoses with FruA_{S.car} and RAMA through the use of glyceraldehydes and a series of aliphatic aldehydes as acceptors. RAMA demonstrated similar activity as FruA_{S.car} under the reaction conditions. The absence of hydroxyl functions for aliphatic aldehydes explains relatively low yields. Nevertheless, the one-pot system avoids the use of expensive DHAP and, cheap starting material DL-glycerol 3-phosphate was adopted to greatly decrease the synthetic cost. Considering the broad applications of FruA in synthetic chemistry, we believe this approach could ultimately contribute to the synthesis of other carbohydrates and their derivatives with biological functions.

1. Experimental section

1.1 Bacterial strains, plasmids, and materials

Oligonucleotide primers were synthesized at Invitrogen (CA, USA). Pfx DNA polymerase was purchased from Invitrogen. Restriction enzymes and T4 ligase were purchased from Fermentas (MBI, Canada). *Escherichia coli* DH5 α [*lacZ* Δ M15 *hsdR* *recA*] was purchased from Gibco-BRL (Gaithersburg, MD). *E. coli* BL21 (DE3) [*F*[−] *ompT* *hsdSB*(*r*_B[−] *m*_B[−]) *gal dcm* (DE3)] and plasmid pET-28a were purchased from Novagen (Carlsbad, CA). Plasmid pKKfda containing *fda* gene was a kind gift from professor Wolf-Dieter Fessner. DL-glycerol 3-phosphate magnesium salt, D-glyceraldehyde, acetaldehyde, propionaldehyde, butyraldehyde, pentanaldehyde, glycerol phosphate oxidase, catalase, aldolase from rabbit muscle (RAMA), acid phosphatase from sweet potato, isopropyl-1-thio- β -D-galactopyranoside (IPTG), imidazole, kanamycin from Sigma-Aldrich (St. Louis, MO). L-glyceraldehyde was purchased from Capot Chemical Co (Hangzhou, China). Ni²⁺-NTA column was purchased from Qiagen (Hilden, Deutschland). Amicon Ultra centrifugal filter (10 K) was purchased from Millipore (Billerica, MA). Bio gel P-2 gel was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). Pierce BCA Protein Assay Kit was purchased from Thermo Scientific (IL, USA).

1.2 One-pot four-enzyme synthesis of D-fructose (or L-sorbose) with FruA_{S.car} using D-glyceraldehyde (or L-glyceraldehyde) as the acceptor

To a solution of DL-glycerol 3-phosphate magnesium salt (548.78 mg, 2.4 mmol) in 6.86 mL ddH₂O was added D-glyceraldehyde (or L-glyceraldehyde) (2 mL, 0.5 M, 1.0 mmol) at pH 7.0, glycerol phosphate oxidase (70 U, 2 mg), catalase (1000 U, 1.18 3L) and FruA_{S.car} (final concentration 0.5 mg/mL). ddH₂O was added to bring the total volume to 10 mL if necessary. The mixture was shaken at rt. for 22 h and the reaction was monitored by TLC (developed by nBuOH/AcOH/H₂O: 2/1/1 (v/v/v) and stained with anisaldehyde sugar stain). The pH was then adjusted to pH ~5 with 6 N HCl and 11 3L acid phosphatase (18 U) was added and the mixture was shaken at 37 °C for 24 h. After cooling to rt., the pH was adjusted to 7.0 with 1 N NaOH and the mixture was diluted with methanol. The solution was filtered through Celite and washed with methanol. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/iPrOH/H₂O: 9/3/1 (v/v/v)) to afford a pale yellow syrup which was further purified by Bio gel P-2 column. After purification, 108 mg D-fructose (60% yield based on D-

glyceraldehyde) and 50.9 mg L-sorbose (28% yield based on L-glyceraldehyde) were obtained respectively.

1.3 One-pot four-enzyme synthesis of ketoses with FruA_{S.car} and RAMA using aliphatic aldehydes

To a solution of DL-glycerol 3-phosphate magnesium salt (457.32 mg, 2 mmol) in 6.86 mL ddH₂O was added corresponding aliphatic aldehyde (1.5 mmol) at pH 7.0, glycerol phosphate oxidase (70 U, 2 mg), catalase (1000 U, 1.18 3L) and FruA (FruA_{S.car} or RAMA) (final concentration 0.5 mg/mL). For pentanaldehyde, 1 mL DMSO was added to the reaction mixture. ddH₂O was added to bring the total volume to 10 mL if necessary. The mixture was shaken at rt. for 22 h and the reaction was monitored by TLC (developed by *n*BuOH/AcOH/H₂O: 2/1/1 (v/v/v) and stained with anisaldehyde sugar stain). The pH was then adjusted to pH ~5 with 6 N HCl and 11 3L acid phosphatase (18 U) was added and the mixture was shaken at 37 °C for 24 h. After cooling to rt., the pH was adjusted to 7.0 with 1 N NaOH and the mixture was diluted with methanol. The solution was filtered through Celite and washed with methanol. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (Dichloromethane/Methanol: 20/1 (v/v)) to afford each pure product. Yields were calculated based on L-glycerol 3-phosphate (1 mmol) since aliphatic aldehydes were used in excess.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Goldberg RN, Tewari YB. J Phys Chem Ref Data. 1995; 24:1669–1698.
- Fessner WD, Helaine V. Curr Opin Biotechnol. 2001; 12:574–586. [PubMed: 11849940]
- Gefflaut T, Blonski C, Perie J, Willson M. Prog Biophys Mol Biol. 1995; 63:301–340. [PubMed: 8599032]
- Bednarski MD, Simon ES, Bischofberger N, Fessner WD, Kim MJ, Lees W, Saito T, Waldmann H, Whitesides GM. J Am Chem Soc. 1989; 111:627–635.
- Brackamp HP, Kula MR. Tetrahedron Lett. 1990; 31:7123–7126.
- Brackamp HP, Kula MR. Appl Microbiol Biotechnol. 1990; 34:287–291.
- Schoevaart R, Van Rantwijk F, Sheldon RA. Tetrahedron: Asymmetry. 1999; 10:705–711.
- Breuer M, Hauer B. Curr Opin Biotechnol. 2003; 14:570–576. [PubMed: 14662384]
- Sugiyama M, Hong Z, Greenberg WA, Wong CH. Bioorg Med Chem. 2007; 15:5905–5911. [PubMed: 17572092]
- Sanchez-Moreno I, Garcia-Garcia JF, Bastida A, Garcia-Junceda E. Chem Commun (Cambridge, U K). 2004:1634–1635.
- Schumperli M, Pellaux R, Panke S. Appl Microbiol Biotechnol. 2007; 75:33–45. [PubMed: 17318530]
- Fessner WD, Sinerius G. Angew Chem, Int Ed. 1994; 33:209–212.
- Li Z, Cai L, Qi Q, Styslinger TJ, Zhao G, Wang PG. Bioorg Med Chem Lett. 2011; 21:5084–5087. [PubMed: 21482110]
- Li Z, Cai L, Qi Q, Wang PG. Bioorg Med Chem Lett. 2011; 21:7081–7084. [PubMed: 22018788]
- Fessner WD, Walter C. Topics in Current Chemistry. 1997; 184:97–194.

16. Schoevaart R, van Rantwijk F, Sheldon RA. Chem Commun (Cambridge, U K). 1999:2465–2466.
17. Kobori Y, Myles DC, Whitesides GM. J Org Chem. 1992; 57:5899–5907.
18. van Herk T, Hartog AF, Schoemaker HE, Wever R. J Org Chem. 2006; 71:6244–6247. [PubMed: 16872211]

Figure 1. SDS-PAGE analysis of FruA_{S.car} expression and purification. Lanes: 1, whole cells not induced; 2, whole cells induced for 20 h; 3, cell lysate; 4, purified FruA_{S.car}; 5, protein marker.

Scheme 1.

One-pot four-enzyme synthesis of ketoses with FruA (FruA_{S,car} and RAMA).

Table 1One-pot four-enzyme synthesis of ketoses with FruA_{S,car} and RAMA

Enzyme	Acceptor	Product	Yield
FruA _{S,car}	D-glyceraldehyde	D-fructose	60%
FruA _{S,car}	L-glyceraldehyde	L-sorbose	28%
FruA _{S,car}	Acetaldehyde	5-deoxy-D-threo-2-pentulose	33%
RAMA	Acetaldehyde	5-deoxy-D-threo-2-pentulose	29%
FruA _{S,car}	Propionaldehyde	5,6-dideoxy-D-threo-2-hexulose	37%
RAMA	Propionaldehyde	5,6-dideoxy-D-threo-2-hexulose	34%
FruA _{S,car}	Butyraldehyde	5,6,7-trideoxy-D-threo-2-heptulose	40%
RAMA	Butyraldehyde	5,6,7-trideoxy-D-threo-2-heptulose	38%
FruA _{S,car}	Pentanaldehyde	5,6,7,8-tetradeoxy-D-threo-2-octulose	23%
RAMA	Pentanaldehyde	5,6,7,8-tetradeoxy-D-threo-2-octulose	13%